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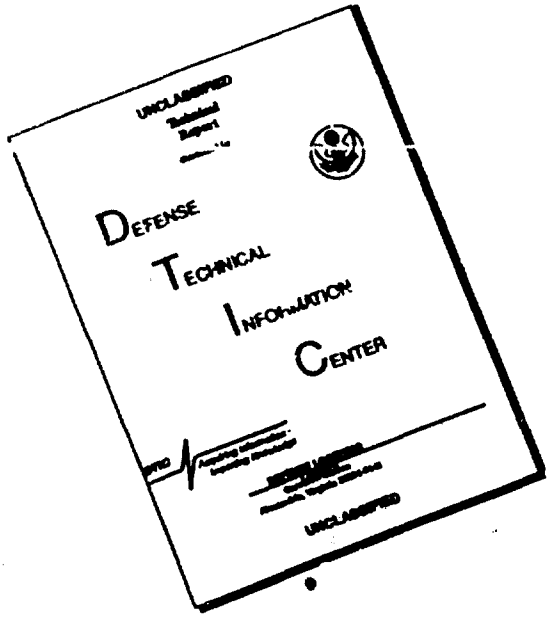

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A STUDY OF THE COMPLETENESS OF PHAGOCYTIC REACTION DURING THE PROCESS OF PLAGUE IMMUNIZATION

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G. G. Korobkov, G. I. Borsuk (Vlasova) and L. M. Samoylova (Irkutsk)

Immunity in the event of vaccination with live Pasteurella pestis of vaccine strain depends to a significant extent on the organism's phagocytic reaction (Zhukov-Verezhnikov, 1940; Pokrovskaya, 1947; Korobkova, 1956; Burrows and Bacon, 1956, et al.).

In the process of immunogenesis a reorganization of the organism's phagocytic reaction takes place. One of the authors of the present work (Vlasova, 1962) established that when animals are immunized by the subcutaneous injection of vaccine strain, the phagocytic number increases (by 50 to 100%) up to the 12th day, then declines somewhat but still remains larger than in control. The percentage of active leukocytes varies somewhat differently: up to the 12th day the number thereof increases insignificantly (10 to 15%), but on the 19th to 21st day after immunization is 20 to 25% greater than the initial level.

In the present investigation we determined the completeness of the phagocytic reaction of the hemoleukocytes and a suspension of liver cells, spleen cells and subcutaneous-tissue cells in white mice during the immunization process according to the methodology of V. M. Berman and Ye. M. Slavskaya (1958, 1959).

For this purpose the mice were injected subcutaneously with 100 million microbe bodies of EV strain of P. pestis. On

the 6th, 11th, 13th, 16th, 19th, 21st and 23rd post-vaccinative day blood and the above-indicated organs and tissues were taken from the mice and the completeness of phagocytic reaction determined for the hemoleukocytes and the cell suspension.

For this purpose the blood was mixed with equal volumes of a 1.5% sodium citrate solution and a 1/1,000,000,000 suspension of EV strain P. pestis. Organs and tissues were excised from the decapitated mouse and bits thereof weighing 100-120 mg were pulverized to a homogeneous mass in a mortar with glass fragments with the addition of 1 ml of physiologic solution: 0.2 ml of the suspension obtained was transferred to a test tube with a microbe suspension containing 500 million M. T. [mikrobnyye tela; microbe bodies] per ml.

After incubation for 30 minutes at 37° two drops of the leukocyte-microbe suspension were distributed in the form of two smears on agar surfaces in a Petri dish. From the smears print preparations were then made on preheated glass slides -- from one smear right after it was made, from the other after incubation for two hours in a Petri dish at 37°. The smear-prints were stained according to Ramonovskiy-Gimza, and the phagocytic number and percentage of active cells calculated.

The live microbes grew inside the leukocytes and in consequence they increased significantly in volume; the dead ones looked like shadows. By comparing the total number of microbes ingested by leukocytes in the first and in the second smear the completeness of phagocytic reaction was judged. Here the indicators obtained from study of the first smear were taken as 100%, while the phagocytic number and percentage of active leukocytes for the second smear were computed as a percentage of the corresponding indicators for the first smear. In the event that the phagocytic number in the second smear was larger than in the first one, which indicated incompleteness of phagocytic reaction, the difference was expressed by a negative number. The data obtained underwent statistical analysis (Tables 1-3). The tables present arithmetic mean $M \pm m$ — standard deviation of calculation.

As can be seen from Tables 1-3, during the immunogenesis process the phagocytic activity of hemoleukocytes, spleen cells, liver cells and subcutaneous-tissue cells varies according to general laws, but not uniformly.

The number of microbes ingested by cells (phagocytic number) decreases in the process of immunogenesis. Especially characteristic in this respect are the experiments involving spleen and liver cells where the number of engulfed microbes,

as compared with initial value, drops 5 to 10-fold. In experiments with subcutaneous-tissue cells the phagocytic number drops less markedly. For hemoleukocytes the phagocytic number increases at first (from the 11th to 21st day after injection of the vaccine) and then drops.

Most characteristic are the variations in the completeness of phagocytosis. For unvaccinated animals completeness of phagocytic reaction was not observed in any of the experiments. Three experiments with spleen cells constitute the exception.

In the case of vaccinated animals for the first two weeks after vaccination there occurred a decline in the degree of completeness of phagocytic reaction, which turned upon microbe propagation in the phagocytes. In later stages -- from the 14th to 25th day -- completeness of phagocytosis by leukocytes and histiocytes of the subcutaneous tissue increases and on the 20th to 25th day complete phagocytosis is observed in most experiments.

In the liver-cell experiments complete phagocytosis was not noted after vaccination. Evidently the large percentage of incomplete phagocytosis in these experiments is explained by the negligible entrapment of microbes by cells. Therefore, even despite the comparatively small extent of microbe propagation the incompleteness of phagocytosis proved to be significant.

In the spleen-cell experiments completeness of phagocytosis as judged by active cells is noted on the 25th day (completeness of phagocytosis as per phagocytic numbers and active leukocytes was observed in other stages as well, but these data are not reliable -- the calculation error is too great, being influenced by the great variability of the values obtained during a small number of observations).

Quite characteristic of the immunogenesis process during plague vaccination are the variations in the percentage of cells where microbe propagation is noted. Whereas in unvaccinated animals the percentage of such cells amounts to 5.5 ± 1.3 in the spleen and to 3.2 ± 0.6 in liver cells, in immunized animals it equals 36 ± 1.5 for the spleen on the 9th post-vaccinative day and 20 ± 4 for the liver on the 12th day; a decrease in the number of such cells sets in then and on the 25th day during the experiment staged on spleen cells it is no longer possible to find a single cell with propagating microbes. The process of increase and subsequent decrease in the percentage of cells with propagating microbes also occurs in subcutaneous-tissue cells and leukocytes.

Table 1

DYNAMICS OF PHAGOCYTIC REACTION OF HEMOLEUKOCYTES IN THE IMMUNOGENESIS PROCESS

1 Показатели фагоцитарной реакции	2 До вакцинации	3 После вакцинации на						4 38-й день
		6	11	13	16	19	21	
5 Фагоцитарное число M ± m	69±1,4	67±12	142±15	69±5,5	67±4	80±7	139±4	77±3
6 Количество клеток с размножающимися микробами M ± m, в %	2,2±0,65	8,5±3	18±2,5	7,6±3,5	2,2±1,3	2±0,5	1,6±0,9	2±0,4
7 Завершенность фагоцитоза по фагоцитарному числу, в %	0	0	0	0	38±13	14±4,5	56,5±9	32±3
8 Опыт 1	−71	−183	−107	−15,7	−32	+	−86	+
" 2	−74	−231	−154	−382	−51	+	+	+
" 3	−16	−107	−105	−67	−24	+	+	+
" 4	−10	−113	−54	−24	+	+	+	+
" 5	+		−19,5	−19	+	+	+	+
9 Число активных клеток	53±1,5	39,7±1,4	39,8±2,3	42±4,7	51,6±2,5	51±1	65,6±3,8	48±0,8
10 Завершенность по активным лейкоцитам	28±6	0	0	35±12	32±5	34,5±7	41±8	32±3

Note: A minus sign before a number indicates that phagocytosis in the case of this particular animal was incomplete; a plus sign, that it was complete

Keys:

1. Indicators of phagocytic reaction
2. Before vaccination
3. Post-vaccinative day
4. 38th day
5. Phagocytic number M ± m
6. Number of cells with propagating microbes M ± m, in %
7. Completeness of phagocytosis as per phagocytic number, in %
8. Experiment
9. Number of active cells
10. Completeness as per active leukocytes

Table 2

DYNAMICS OF PHAGOCYTIC REACTION OF SUBCUTANEOUS-TISSUE CELLS IN THE IMMUNIZATION PROCESS

1 Показатели фагоцитарной реакции	2 До вакцинации	3 После вакцинации на		4
		12	15	25-й день
5 Фагоцитарное число $M \pm m$	90±5	85±5	106±10	56±14
6 Количество клеток с размножающимися микробами $M \pm m$, в %	5,4±0,7	6±0,8	7±1	0
7 Завершенность фагоцитоза по фагоцитарному числу $M \pm m$, в %	0	0	0	37±8,7
8 Опыт 1	−50	−117	−97	−153
" 2	−23	−115	−72	+
" 3	−22	−140	−90	+
" 4	−54	−166	−24	+
" 5	−90		−59	+
9 Число активных клеток	40,4±4,2	49±3,5	47±2,5	32±7
10 Завершенность по активным клеткам	0	0	0	33±6

Note: Same as the Note to Table 1.

Keys:

1. Indicators of phagocytic reaction
2. Before vaccination
3. Post-vaccinative day
4. 25th day
5. Phagocytic number $M \pm m$
6. Number of cells with propagating microbes $M \pm m$, in %
7. Completeness of phagocytosis as per phagocytic number, in %
8. Experiment
9. Number of active cells
10. Completeness as per active cells

Table 3

DYNAMICS OF PHAGOCYTIC REACTION OF LIVER AND SPLEEN CELLS DURING IMMUNIZATION PROCESS

1 Показатели фагоцитарной реакции	2 До вакцинации	3 После вакцинации на 6	9	12	16	19	21	4 25-й день
		5 Клетки печени						
6 Фагоцитарное число M ± m	117±42,5	99±14	86±18	94±35	74±16	28±12	24±2,5	12 не исследовали
7 Количество клеток с размножающимися микробами M ± m, в %	5,5±1,3	16±11	–	20+4	1,6±2	3±1	2±1,4	
8 Завершенность фагоцитоза по фагоцитарному числу	0	0	–	0	20±8,5	0	0	
9 Опыт 1	–19	–19	–178	–100	–80	–342	–267	
" 2	–19	–355	–115	–51	–63	–370	–508	
" 3	–34	–51	–67	–151	+	–162	–812	
" 4	–36	–573	+	–100	+	–475	–741	
" 5	0	–425	+	–133	+	–402	–150	
10 Число активных клеток	48±5,5	49±4,8	–	45±3,5	38±5,5	18±2,7	17±2,5	
11 Завершенность по активным клеткам	0	0	16±13	–	17±15	0	0	

Note: Same as the Note to Table 1.

Keys:

1. Indicators of phagocytic reaction
2. Before vaccination
3. Post-vaccinative day
4. 25th day
5. Liver cells
6. Phagocytic number M± m
7. Number of cells with propagating microbes M± m, in %
8. Completeness of phagocytosis as per phagocytic number, in %
9. Experiment
10. Number of active cells
11. Completeness as per active cells
12. Not investigated

contd

Table 3 (contd)

	13 Клетки селезенки							
6 Фагоцитарное число $M \pm m$	140±43	68±3	84±16	50±13	50±7	16±3	24±11,5	17±4
7 Количество клеток с размножающимся микробами $M \pm m$, в %	3,2±0,6	2,2±1,3	36±1,5	7,2±3,5	0,2±0,2	1,2±	2±0,5	0
8 Завершенность фагоцитоза по фагоцитарному числу	23±5	0	22,7±11,7	21,5±4	56±20	0	0	18,7±8,5
9 Опыт 1	−137	−25	−656	−85	−127	−750	−1154	−10
2	−136	−30	−68	−556	−40	−371	−405	−158
3	+	−142	+	−242	−19	−160	−730	+
4	+	−40	+	+	+	−46	−344	+
5	+	−31	+	+	+	−100	−767	+
10 Число активных клеток	44,7±1,7	37,6±3,5	38±5,5	9±5	27±4,5	13±1,5	11±1	17,2±4,3
11 Завершенность по активным клеткам	15±5,3	0	40±15	±4	33±16	0	0	36±8,5

Note: Same as the Note to Table 1.

Keys:

6. Phagocytic number M ± m
7. Number of cells with propagating microbes M ± m, in %
8. Completeness of phagocytosis as per phagocytic number, in %
9. Experiment
10. Number of active cells
11. Completeness as per active cells
13. Spleen cells

In immunogenesis the role of spleen and liver phagocytes differs significantly from the role of hemoleukocytes and subcutaneous-tissue cells. Subcutaneous-tissue phagocytes and hemoleukocytes are the first to take the "blow" when microbes strike cutaneously or intracutaneously, whereas liver and spleen cells do not become involved in it until the infectious process generalizes.

During the first phase of an infectious process completeness of phagocytic reaction is not the main indicator of the organism's protective reaction since leukocytes with both complete and incomplete phagocytosis form an exudate and detain microbes in their membrane for two or three hours during which time the formation of a connective-tissue barrier begins; as for spleen and liver cells, bacteriostatic effect is the principal form whereby these organs take part in the phagocytic reaction.

In the immunized process which develops after vaccination with avirulent strains of P. pestis great importance evidently attaches to the bacteriostatic effect by virtue of which microbes engulfed by spleen cells remain alive, but the propagative process comes to a halt. This is indicated by the fact that there are no propagating microbes in the spleen cells on the 25th post-vaccinative day although in 40% of the cases phagocytosis in spleen cells is incomplete during these periods.

Conclusions

1. The phagocytic number for spleen and liver RES [reticuloendothelial system] cells declines significantly in the process of immunogenesis with live P. pestis vaccine; on the 21st to 25th day it is five to ten-fold less than in the case of intact animals. The phagocytic number for hemoleukocytes in the immunogenesis process increases on the 11th post-immunization day, then drops to initial level. The phagocytic number for subcutaneous-tissue cells during the immunization process drops 1.5 to two-fold as of the 25th day after vaccination.

2. Completeness of phagocytic reaction in experiments with hemoleukocytes, spleen cells, liver cells and subcutaneous-tissue cells declines greatly on the 6th to 12th post-vaccinative day since P. pestis actively propagates inside the cells during this period. In subsequent periods the completeness of phagocytic reaction rises and on the 21st post-vaccinative day in most experiments phagocytosis becomes complete: 56 ± 9 for hemoleukocytes, 33 ± 6 for subcutaneous-tissue cells,

36 8.5 for spleen cells. In liver cells completeness of phagocytic reaction is not observed.

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